Organic LETTERS

Letter

pubs.acs.org/OrgLett

# Pseudellones A–C, Three Alkaloids from the Marine-Derived Fungus *Pseudallescheria ellipsoidea* F42–3

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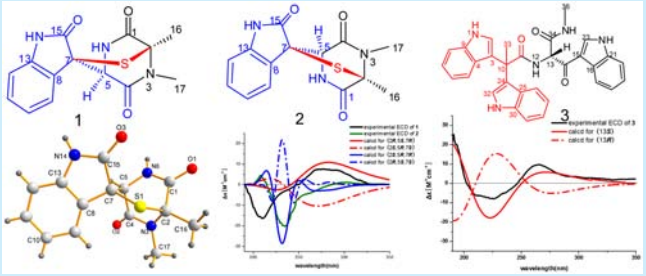
Supporting Information

**ABSTRACT:** Pseudellones A and B (**1** and **2**), a pair of irregularly bridged epimonothiodiketopiperazine diastereomers constructed from unusual 3-indolylglycine and alanine residues, and an alkaloid pseudellone C (**3**) possessing a unique skeleton were isolated from the marine-derived fungus *Pseudallescheria ellipsoidea* F42–3. Their structures were determined by spectroscopic data, ECD calculation, and X-ray single crystal diffraction. The biogenetic pathways of **1**–**3** were proposed, and 1*H*-indole-3-carboxylic acid (**4**), a plausible biosynthetic intermediate, was coisolated.



Marine-derived fungi have become a rich source of structurally unique and biologically significant metabolites.[1] In recent years, we investigated the secondary metabolites from fungi associated with marine invertebrates, such as the soft coral *Sarcophytum tortuosum*, the starfish *Acanthaster planci*. A variety of new and bioactive compounds were obtained.[2] In our continued search for a new source of marine-derived fungi for novel chemistry, soft corals of the genus *Lobophytum* attracted our attention. These corals are abundant in the South China Sea, and a large number of bioactive substances were isolated from them.[3,4] However, a supply issue has become a serious obstacle to the ultimate development of these bioactive substances. Fungi associated with soft corals of the genus *Lobophytum* represent a new alternative source of novel bioactive molecules.

In a preliminary study, about 100 fungi were isolated and purified from the soft coral *Lobophytum crassum* collected in Hainan Sanya National Coral Reef Reserve, China. A fungal strain authenticated as *Pseudallescheria ellipsoidea* by an ITS-rDNA sequence grows fast in glucose-peptone-yeast (GPY) medium. Research on the fungus *Pseudallescheria ellipsoidea* has been seldom reported. As for the metabolites, only two new antifungal antibiotics have been isolated from this fungus.[5,6] Additionally, the $^1$H NMR spectrum of the EtOAc extract of the culture broth displaying $D_2O$ unchangeable aryl proton signals in the region of 6.80–8.50 ppm in DMSO-$d_6$ solvent, especially the relatively rare signals at $\delta_H$ 8.00–8.50, indicated the high discovery rates of novel chemistry. By NMR-guided chemical studies of the fungus *Pseudallescheria ellipsoidea*, a pair of unprecedented epimonothiodiketopiperazine diastereomers, pseudellones A and B (**1** and **2**) characterized by a monosulfide bridge joining between the $\beta$- and $\alpha$-positions of unusual 3-indolylglycine and alanine residues, an alkaloid pseudellone C (**3**) possessing a unique skeleton with a 2,2-di(3-indolyl)-1-propone fragment attached to a tryptophan derivative via an amide bond, and 1*H*-indole-3-carboxylic acid (**4**), a putative biogenetic intermediate (Figure 1), were obtained. Herein we report the details of structure elucidation, plausible biosynthetic pathways, and antibacterial evaluation of compounds **1**–**3**.

The molecular formula of pseudellone A (**1**) was revealed to be $C_{14}H_{13}N_3O_3S$ by HR EIMS at $m/z$ 303.0668 $[M]^+$, requiring

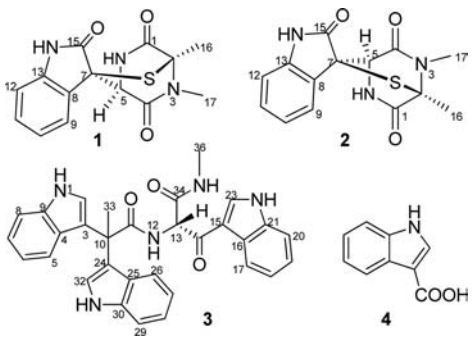


**Figure 1.** Structures of compounds **1**–**4**.

**Received:** August 9, 2015
**Published:** October 9, 2015





DOI: 10.1021/acs.orglett.5b02311
*Org. Lett.* 2015, 17, 5156–5159

Table 1. $^{1}H$ and $^{13}C$ NMR Data of Compounds 1−3 at 400/100 MHz, $\delta$ in ppm

| | 1[a] | | 2[a] | | 3[b] | | | 3[b] | |
|---|---|---|---|---|---|---|---|---|---|
| no. | $\delta_C$, mult. | $\delta_H$, mult. ($J$, Hz) | $\delta_C$, mult. | $\delta_H$, mult. ($J$, Hz) | $\delta_C$, mult. | $\delta_H$, mult. ($J$, Hz) | no. | $\delta_C$, mult. | $\delta_H$, mult. ($J$, Hz) |
| 1 | 167.3, C | | 167.1, C | | NH | 10.23 (s) | 20 | 122.6, CH | 8.14, dt (8.0, 0.8) |
| 2 | 68.1, C | | 68.2, C | | 124.5, CH | 7.25, d (2.8) | 21 | 137.7, C | |
| 3 | | | | | 119.7, C | | 22 | NH | 11.24 (s) |
| 4 | 167.1, C | | 168.0, C | | 126.9, C | | 23 | 136.8, CH | 8.48, d (3.2) |
| 5 | 61.1, CH | 4.17, d (4.4) | 60.9, CH | 4.05, d (5.6) | 112.6, CH | 7.41, dt (8.4, 0.8) | 24 | 119.7, C | |
| 6 | NH | 9.07, d (4.8) | NH | 9.49, d (5.6) | 122.1, CH | 7.03, td (7.2, 0.8) | 25 | 127.0, C | |
| 7 | 58.2, C | | 59.9, C | | 119.5, CH | 6.82, td (8.0, 1.2) | 26 | 112.7, CH | 7.46, dt (8.0, 0.8) |
| 8 | 127.2, C | | 127.7, C | | 121.7, CH | 7.37, brd (8.4) | 27 | 122.2, CH | 7.08, td (7.2, 0.8) |
| 9 | 125.7, CH | 6.89, dd (6.8, 0.8) | 125.3, CH | 7.22, dd (7.6, 0.8) | 138.5, C | | 28 | 119.6, CH | 6.93, td (8.0, 0.8) |
| 10 | 129.5, CH | 7.24, td (7.6, 1.6) | 129.6, CH | 7.29, td (7.6, 1.2) | 48.0, C | | 29 | 121.4, CH | 7.47, ddd (7.6, 1.6, 0.8) |
| 11 | 121.6, CH | 6.97, td (7.6, 0.8) | 122.0, CH | 7.046, td (7.6, 1.2) | 175.5, C | | 30 | 138.4, C | |
| 12 | 109.9, CH | 6.87, d (7.6) | 110.0, CH | 6.89, d (7.6) | NH | 7.58, d (6.0) | 31 | NH | 10.28 (s) |
| 13 | 141.3, C | | 141.1, C | | 62.5, CH | 5.69, d (6.4) | 32 | 125.1, CH | 7.28, d (2.4) |
| 14 | NH | 10.77 (s) | NH | 10.76 (s) | 187.1, C | | 33 | 26.2, $CH_3$ | 2.14 (s) |
| 15 | 174.1, C | | 173.9, C | | 115.1, C | | 34 | 168.1, C | |
| 16 | 26.1, $CH_3$ | 3.06 (s) | 26.3, $CH_3$ | 2.93 (s) | 127.1, C | | 35 | NH | 6.46, dd (8.0, 4.0) |
| 17 | 16.4, $CH_3$ | 1.77 (s) | 16.2, $CH_3$ | 1.76 (s) | 112.9, CH | 7.50, dt (7.6, 0.8) | 36 | 26.2, $CH_3$ | 2.46, d (4.8) |
| 18 | | | | | 124.2, CH | 7.22, td (7.2, 1.6) | | | |
| 19 | | | | | 123.1, CH | 7.17, td (7.2, 0.8) | | | |

[a]Measured in DMSO-$d_6$. [b]Measured in Acetone-$d_6$.

10 degrees of unsaturation. The $^{13}C$ NMR and DEPT spectra (Table 1) displayed seven quaternary carbons, four $sp^2$ methines, one $sp^3$ methine, and two methyls. In the $^{1}H$ NMR spectrum, two exchangeable protons at $\delta_H$ 10.77 and 9.07 attributable to the amide protons, four aromatic protons at $\delta_H$ 6.87 (d, H-12), 6.97 (td, H-11), 7.24 (td, H-10), 6.89 (dd, H-9), and one methine proton at $\delta_H$ 4.17 (d, H-5) and two methyl singlets at $\delta_H$ 1.77 (s, $H_3$-16), 3.06 (s, $H_3$-17) were observed. A 1,2-disubstituted benzene nucleus was deduced from the four intercoupling aromatic proton signals in conjunction with the $^{1}H-^{1}H$ COSY correlations from H-9 to H-10, H-10 to H-11, and H-11 to H-12, and HMBC cross peaks of H-9 and H-10 with C-8, H-11 and H-12 with C-13. The correlations of H-9 with C-7 and H-14 with C-13, C-15, C-8, and C-7 in the HMBC spectrum indicated that the quaternary carbon C-7 and amide amino group NH-14 were attached to the C-8 and C-13, respectively, and formed an indolin-2-one moiety. This connectivity matched well with the chemical shift pattern of indolin-2-one. Furthermore, the $^{1}H$ and $^{13}C$ NMR spectra of **1** displayed characteristic signals for a diketopiperazine. Two amide carbonyls at $\delta_C$ 167.3 and 167.1 and two $\alpha$-amino acid carbon resonances at $\delta_C$ 61.1 and 68.1 implied the presence of two amino acid residues. The $^{1}H-^{1}H$ COSY correlation between H-5 and H-6 and the HMBC couplings from H-6 to C-1, C-2, and C-4, H-5 to C-4, $H_3$-17 to C-4 and C-2, and $H_3$-16 to C-2 and C-1 (Figure 2) suggested the diketopiperazine ring was constructed from glycine and alanine residues with a methyl group substituted at the N-3 position. The C-5 of the diketopiperazine fragment was connected to C-7 by the $^{1}H-^{13}C$ coupling of H-5 with C-7. One indolin-2-one and one diketopiperazine ring accounted for 9 out of 10 sites of unsaturation, so compound **1** was inferred to contain an additional ring. Therefore, the remaining sulfur atom must attach to the C-2 and C-7 positions to form another ring to satisfy the molecular formula and complete its planar structure.

The absence of characteristic protons on the chiral carbons of **1** creates difficulties in determining the relative configurations by NOESY. It was noteworthy that the cage rigidity for

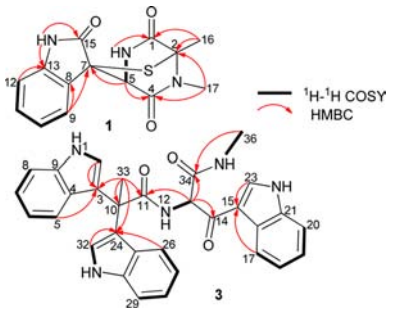



Figure 2. $^{1}H-^{1}H$ COSY and key HMBC correlations of **1** and **3**.

the existence of the sulfide bridge only permitted four stereochemical options. To elucidate the stereochemistry, the ECD spectra of compound **1**, with the four feasible configurations (2*R*, 5*S*, 7*R*), (2*S*, 5*R*, 7*S*), (2*S*, 5*R*, 7*R*), and (2*R*, 5*S*, 7*S*), were calculated and compared with the experimental ECD spectrum [CD ($c$ 0.05, MeCN) ($\Delta\varepsilon_{max}$) 210 (−16.40), 276 (+7.58)]. As illustrated in Figure 3, the calculated ECD curve matched the experimental one for the (2*R*, 5*S*, 7*R*)-configuration. Thus, the absolute configuration of compound **1** was established as (2*R*, 5*S*, 7*R*). By extensive effort, the single crystals were successfully grown in methanol solvent. The absolute configuration of **1** was ambiguously determined as (2*R*, 5*S*, 7*R*) with the Flack parameter value −0.003(15) by single-crystal X-ray diffraction analysis (Figure 4) using Mo K$\alpha$ radiation.

Pseudellone B (**2**) was isolated as a white solid. The molecular formula was established to be $C_{14}H_{13}N_3O_3S$ based on the HR ESIMS peak at $m/z$ 326.0574 $[M + Na]^+$. The overall NMR spectroscopic profile (Table 1) of **2** closely resembled that of **1** except that the chemical shift of C-7 was shifted downfield by 1.7 ppm in comparison to the corresponding carbon of **1**. The planar structure of **2** was confirmed by the splitting pattern and coupling constants of four aromatic protons and the HMBC data. Detected at 254 nm of UV absorption wavelength, the HPLC analysis of the



DOI: 10.1021/acs.orglett.5b02311
Org. Lett. 2015, 17, 5156−5159

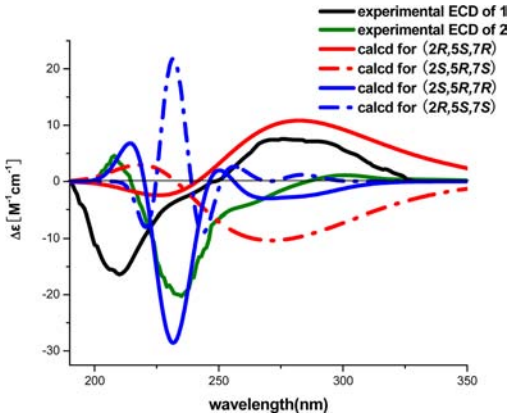



**Figure 3.** Comparison of the experimental ECD spectra of **1** and **2** with the calculated ECD spectra for four stereochemical options.

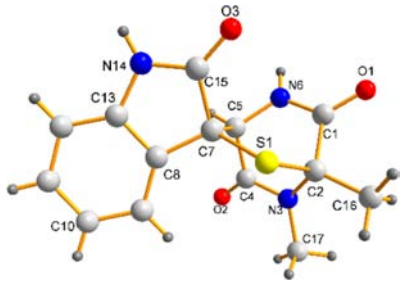



**Figure 4.** ORTEP drawing of compound **1**.

mixture of compounds **1** and **2** furnished two separable peaks with the retention time at 7.34 and 6.78 min (Figure S1). It was indicated that **2** was the stereoisomer of **1**. The absolute configuration was assigned as (2*S*, 5*R*, 7*R*) by comparison of the experimental and calculated ECD spectra. The experimental CD curve [CD (*c* 0.05, MeCN) ($\Delta\varepsilon_{max}$) 208 (+4.59), 235 (−20.26)] was consistent with the calculated ECD spectrum for (2*S*, 5*R*, 7*R*) (Figure 3).

Pseudellone C (**3**) was obtained as a pale yellow solid. The molecular formula was established to be $C_{31}H_{27}N_5O_3$ as deduced by HR ESIMS at *m*/*z* 540.2016[M + Na]$^+$, corresponding to 21 degrees of unsaturation. The $^{13}$C NMR and DEPT spectra (Table 1) displayed 31 carbons, consisting of 3 carbonyls, 9 sp$^2$ quaternary carbons, 1 sp$^3$ quaternary carbon, 15 sp$^2$ methines, 1 sp$^3$ methine, and 2 methyls. The $^1$H NMR spectrum showed five $D_2O$-exchangeable proton signals. By careful inspection of 1D NMR data, the presence of three indole nuclei were deduced from chemical shifts and the splitting pattern of three sets of five aromatic protons in conjunction with chemical shifts of three sets of eight aromatic carbons. This deduction was further confirmed by the cross peaks of H-5 with H-6, H-6 with H-7, and H-7 with H-8 in the $^1$H−$^1$H COSY spectrum and HMBC correlations from H-2 and H-5 to C-3. Similar $^1$H−$^1$H COSY and HMBC correlations were also observed in the other two indole rings. Additionally, three exchangeable protons at $\delta_H$ 11.24 (s), 10.28 (s), and 10.23 (s) were due to the typical NH protons of three indole rings. Three indole fragments together with three carbonyls accounted for 21 degrees of unsaturation, so the remaining resonance signals should be acyclic. The connectivity of three indole rings and the remaining structure units were determined by $^1$H−$^1$H COSY correlations between H-12 and H-13 as well as between H-35 and H-36 and $^1$H−$^{13}$C couplings from $H_3$-33 to C-10, C-3, C-24 ,and C-11, H-13 to C-14, C-34, and C-11, and $H_3$-36 to C-34 in the HMBC spectrum (Figure 2), which finally completed the planar structure of compound **3**.

The experimental CD spectrum [CD (*c* 0.03, MeCN) ($\Delta\varepsilon_{max}$) 226 (−8.01), 266 (+9.76)] of **3** was consistent with the computed ECD curve of the 13*S* configuration shown in Figure 5. Therefore, the absolute stereochemistry of compound **3** was assigned as 13*S*.

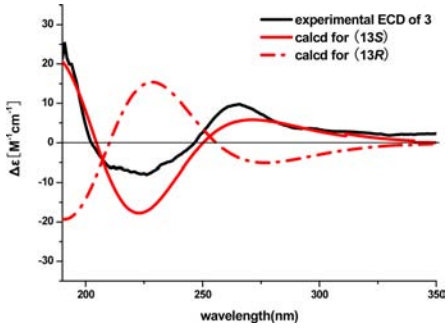
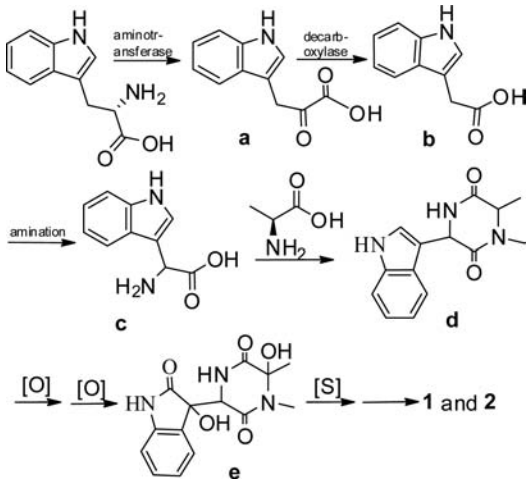



**Figure 5.** Comparison of the experimental ECD and those calculated ECD spectra for (13*S*) and (13*R*) of **3**.

Pseudellones A (**1**) and B (**2**), having an unprecedented monosulfide bridge joining the *β*-position and *α*-positions of amino acid residues, belong to epipolythiopiperazines. Furthermore, pseudellones A and B possessed a diketopiperazine ring built from unusual 3-indolylglycine and alanine. 3-Indolylglycine is a nonproteinogenic amino acid, which was only reported as a synthetic product.[7] The biogenesis of pseudellones A and B was proposed via the amino acid pathway (Scheme 1). Tryptophan aminotransferases convert tryptophan

**Scheme 1. Plausible Biosynthetic Route of 1 and 2**




to indole-3-pyruvic acid (**a**), and then decarboxylases participate in transforming **a** to indole-3-acetic acid (**b**),[8] which was subsequently aminated to furnish 3-indolylglycine (**c**). Further intermolecular condensation between **c** and alanine afforded the diketopiperazine **d**, which was followed by oxidation, sulfurization, elimination, and cyclization to generate compounds **1** and **2**.

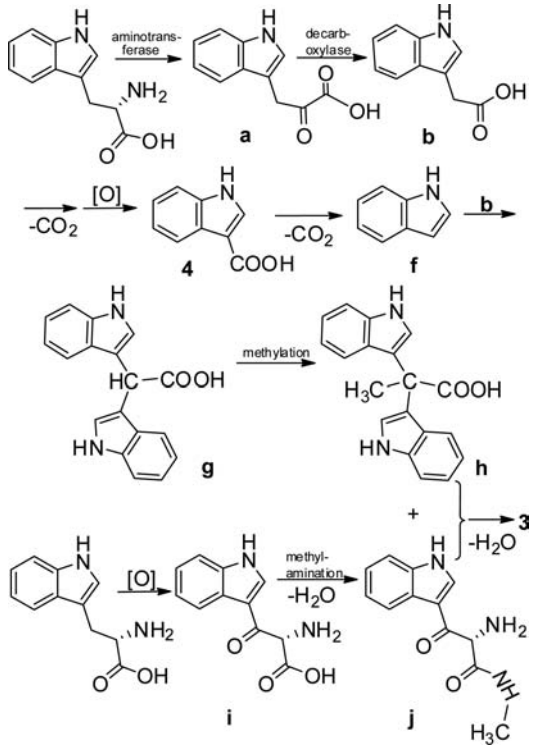
Pseudellone C (**3**) possessed a unique skeleton with a 2,2-di(3-indolyl)-1-propone fragment attached to a tryptophan derivative via an amide bond. The 2,2-di(3-indolyl)-1-propone



DOI: 10.1021/acs.orglett.5b02311
*Org. Lett.* 2015, 17, 5156−5159

unit was also possessed by 2,2-bis(3,3′-indolyl) propionic acid from *E. coli*.[9] However, this unit was linked to an additional tryptophan derivative via an amide bond for pseudellone C, different from 2,2-bis(3,3′-indolyl) propionic acid. From the biosynthetic aspect, compound **3** was also derived from tryptophan. The biogenetic route is shown in Scheme 2. Very

**Scheme 2. Plausible Biosynthetic Route of 3**




fortunately, the plausible biosynthetic intermediate 1*H*-indole-3-carboxylic acid (**4**) was isolated and recrystallized as single crystals (Figure 6) in methanol for X-ray diffraction analysis, which further validated the proposed biogenetic route to a certain extent.

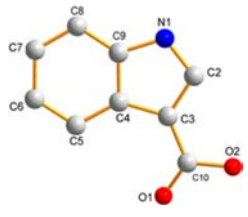


**Figure 6.** ORTEP drawing of compound **4**.

Many epipolythiodiketopiperazines exhibited antibiotic or antitumor activity.[10] However, pseudellones A and B did not display significant antibacterial activity against the Gram-positive *Staphylococcus aureus* (ATCC29213), methicillin-resistant *Staphylococcus aureus* (R3708), and the Gram-negative *Escherichia coli* (ATCC25922) at the 50 $\mu$M concentration. This result indicated that the location of sulfide linkage probably influences the bioactivity.

Compounds **1**−**4** are strikingly different from the secondary metabolites from the host soft coral *Lobophytum crassum*.[4] Overall, the discovery of pseudellones A−C gives further evidence that fungi associated with soft corals of the genus *Lobophytum* represent great potential for producing novel chemistry.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02311.

X-ray crystallographic data for **4** (CIF)
X-ray crystallographic data for **1** (CIF)
Experimental section, MS, 1D and 2D NMR spectra of all compounds (PDF)

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### Author Contributions

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### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

This project is financially supported by Guangdong Provincial Science and Technology Research Program (Nos. 2013B021100010, 2013B021100012, 2014A020217004, and 2015A020216007), Guangzhou Science and Technology Research Program (No. 2014J4100059), and the Fundamental Research Funds for the Central Universities (Nos. 15ykpy05 and 14yksh01).

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DOI: 10.1021/acs.orglett.5b02311
*Org. Lett.* 2015, 17, 5156−5159